REMARKS

All the pending elected claims have been rejected on both 35 U.S.C. 101 ground for lack of patentable utility and 35 U.S.C. 112 ground for lack of enablement.

Applicant respectfully traverses the rejection for the following reasons:

In the 101 rejection, the Examiner faults the application for discussing the literature and merely reciting hypothetical benefits without providing experimental evidence. However, the manner in which an invention is arrived at, whether through experimentation or by reviewing the prior art and drawing a new and non yet discovered and appreciated conclusion from it is irrelevant. What really matters is whether the invention presents a useful tool that contributes to the progress of science.

In the 112 rejection, the Examiner opines that, because the invention lacks patentable utility, the determination of efficacious treatment parameters would require a high amount of experimentation.

Applicant submits that these observations overlook or misinterpret a large part of the disclosure, and points out the following remarks that demonstrate the utility of the invention and its manner of enablement under the following topics:

Examples of Mitochondrial Protection and Opportunities for Comprehensive Treatment of Disease Mechanisms;

Manner of Deployment of the Instant Patent's Technology to Optimize Drugs and their Development;

Toxicology, Safety Trials and Efficacy Clinical Trials in Man; and

Formulation - Dosage, Chemical Forms, Routes of Delivery and Pharmaceutical Compositions.

Examples of Mitochondrial Protection and Opportunities for Comprehensive Treatment of Disease Mechanisms

The central topic of this invention is that a range of polyamine compositions protect mitochondria during neurodegenerative and diabetic diseases as demonstrated previously by the prevention of Parkinson induced toxicity by the mitochondrial toxin 1,-methyl, 4-phenyl, 1,2,3,6 tetrahydropyridine (MPTP) in the parent U.S. Patent No. 5,906,996. See also U.S. Patent No. 6,576,672. Polyamine Treatment of Neurological Disorders. June 10, 2003 Murphy, and subsequently prevention of mitochondrial damage by an array of mitochondrial neurotoxins and diabetogenic toxins as described in a continuation in part of the instant application Composition, Synthesis and Therapeutic Application of Polyamines December 18, 2002 filed as a P.C.T. application PCT/US02/40732 with domestic entry in the U.S. Patent Application No. 10/499,931.

The basic opportunity of this invention is that it allows the medicinal chemist and physician to treat a broad group of diseases, both neurodegenerative and diabetic, which group of diseases and their complications involve mitochondrial damage, said mitochondrial damage occurring in the preclinical and early clinical stages of neurodegenerative and diabetic diseases. Currently mitochondrial damage is not treatable by other families of compounds.

However the invention has a very significant further impact in providing more specific and comprehensive treatment of many other known biochemical aspects of neurodegenerative and diabetic diseases alongside the initial mitochondrial damage. The medicinal chemist can design

drugs which treat mitochondrial damage whilst by means of the same molecule can simultaneously address any one or more or all of a group of biochemical deficits which occur at various times during the progression and during the development of complications in neurodegenerative diseases and diabetes. Likewise the physician can elect to treat the patient in the preclinical and clinical stages of disease selectively according to which biochemical components need to be regulated alongside the mitochondrial component. Such a customized approach is now all the more relevant as diagnosis of mitochondrial DNA damage during the incipient and clinical stages of disease is practicable and when as in diabetes a physician may track more than one hundred laboratory parameters in following the clinical evolution of diabetic disease in a patient or clinical trial subject. Mitochondrial DNA based diagnosis of these acquired mitochondrial DNA diseases and the inherited mitochondrial DNA diseases is routinely practicable in laboratories such as that of Dr. Douglas Wallace at University of California Irvine, requiring a drop of peripheral blood pcr amplification and thirty six hours of technician or machine time to process the sample. Mass applicable diagnostic tests of mitocondrial DNA damage and other biochemical defects in the diabetes can come to market at any time and be retailed in the single or double digit dollar price range. In essence this invention provides opportunities for specific and comprehensive single drug treatments for these diseases in a medical practice environment where personalized medicine defines treatment in terms of addressing the biochemical defects rather than a one hat fits all approach based upon treating patients according to a disease label. When combined with longitudinal medical care the fruits of this invention can enhance the duration and quality of life of neurodegenerative disease and diabetic patients by preventing progression and complications of disease.

Prior to the invention of the instant patent application and its parent U.S. Patent No.

5,906,996, it was not practicable by means of other chemical families to treat multiple biochemical mechanisms of a disease process simultaneously by means of a single drug compound. For example no one had devised a molecule which was simultaneously a tyrosine phosphatase inhibitor (insulin sensitizing agent) and a protein kinase C inhibitor. Inhibition of protein kinase C is a key target towards the treatment and prevention of diabetic renal damage and diabetic peripheral neuropathy. Similarly no compound family was available which would prevent mitochondrial damage and simultaneously inhibit acetylcholinesterase to treat Alzheimer's disease.

Based on the prior art related to common mitochondrial diseases discussed in pages 14 – 21 and the examples of biochemical disease mechanisms cited in the instant patent application on pages 62 – 65 it is evident to either a drug designer or physician prescribing such drugs that there are an array of therapeutic choices from which to customize individual patients' treatments, in accordance with the specific sub-varieties of disease patients' suffer from.

Page 62 of the instant patent application describes how an effective Alzheimer treatment, involving mitochondrial DNA protection by means of actions a), b), c) and d) and a range of other actions e) to s) inclusive, can be designed by creating a compound which has activity in all of the actions from a) to s) inclusive and an effective early stage Parkinson treatment can be created by designing a compound effective towards actions a) to m) inclusive and q) to s) inclusive, and a later stage Parkinson treatment would as with the Alzheimer treatment include activity related to all of the actions from a) to s) in the compound.

Examples in relation to diabetic diseases is discussed in pages 62 – 65. Diabetic Diseases include a range of about forty biochemical errors. Mitochondrial DNA damage and mitochondrial malfunction are linked to both type I and Type II diabetes via toxic models such as streptozotocin

and alloxan and exposure to environmental agent such as herbicides, agent orange. The current treatment of diabetes is limited by mitochondrial malfunction even when hyperglycemia is controlled by insulin and or oral drugs. The instant patent application introduces polyamine compositions, which prevented three toxic models of diabetes including streptozotocin, alloxan and diazoxide. These models cause mitochondrial damage by inhibition of the mitochondrial DNA enzyme poly ADP ribose phosphatase (PARP) and the mitochondrial enzyme glycerol 3-phosphatase dehydrogenase.

Core biochemical defects in diabetes and its cardiovascular complications are:

Mitochondrial DNA damage;

Impaired adenosine triphosphate (ATP) production in consequence of mitochondrial damage;

Impaired exocytosis of insulin resulting from reduced ATP levels;

Altered Fat Metabolism;

Altered Body Mass Index; and

Inappropriate ratio of tyrosine kinase to tyrosine phosphatase activity.

Thus the central thrust of drug development offered by this invention is to protect mitochondria, thereby maintaining ATP production, thereby stimulating exocytosis of insulin and increasing insulin sensitivity by inhibiting tyrosine phosphatase enzyme. These actions normalize glucose and fat metabolism, and body mass index.

As described below Applicant designed polyamine compounds based on structure activity relationships to accomplish these actions and further address design to control biochemical actions relevant to microvascular and macrovascular and neurological complications.

Thus the treatment of diabetic diseases can benefit from compounds which protect

mitochondrial function alongside addressing a selected range of defects relevant to the subvariety of diabetes. Today diabetes is evaluated in terms of pathogenesis and severity by more then a hundred biochemical measurements alongside clinical evaluation. Historically pharmaceuticals have been developed and utilized with a view to treating diabetes with relatively little attention to disease subtype or by means of polypharmacy to treat several biochemical events.

The biochemical actions of polyamine drugs to treat diabetes, which can be optimized from this invention are:

a) Competitive inhibition of uptake of xenobiotics at the polyamine transport site, such organic molecules being a cause of mitochondrial DNA damage; b) Steric shielding of DNA from organic molecules by compacting DNA; c) Limitation of mitochondrial DNA damage by removal of free copper, iron, nickel, mercury and lead ions by the presence of a polyamine; d) Induction of metallothionein gene transcription; e) Inhibition of protein kinase C; f) Mitochondrial reuptake of calcium; g) Binding and conservation of reduced glutathione; h) Induction of ornithine decarboxylase by glutathione; i) Maintenance of the homeostasis of the redox environment; j) Inhibition of superoxide dismutase, amine oxidase by binding of free copper; k) stimulate release of insulin by promoting exocytosis; l) enhance glucose tolerance and decrease blood cholesterol and triglycerides, and increase high density lipoprotein; m) decrease P-enolpyruvate carboxykinase (PEPCK) transcription, thus decreasing gluconeogenesis; n) decrease tyrosine aminotransferase gene expression; o) increase expression of glucokinase gene; p) induce pyruvate kinase; q) decrease mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCoAS) gene expression; r) decrease the expression of the liver and pancreas glucose-transporter GLUT-2 gene in diabetic animals to the level seen in controls; s) increase

the amount of the insulin-sensitive glucose transporter, GLUT4 by stimulating its transcription; t) inhibition of protein tyrosine phosphatases (PTP); u) suppress nitric oxide production by macrophages; v) positive cardiac inotropic effect; w) restore albumin mRNA levels in diabetic animals by increasing hepatic nuclear factor 1 (HNF 1); x) it restores triiodothyronine T_3 levels; y) decrease protein kinase C activity; z) PPARα and PPARγ agonism or antagonism.

Within this large group of biochemical errors the Applicant recognized that polyamine compounds provide opportunity to design compounds and complexes containing the two metals chromium and vanadium, effective in protecting mitochondrial DNA and simultaneously having efficacy on any selected subgroup or all of these actions. Indeed reverse effects are necessary in different treatment situations, as for example, a juvenile type I diabetes would benefit from being treated with a compound which exhibits PPARγ antagonism whereas a maturity onset diabetes of the young would benefit from a compound which exhibits PPARγ agonism.

Specific but non-limiting examples illustrate some of the many possibilities for polyamine treatments of neurodegenerative and diabetic diseases, which are evident from this invention on pages 62 - 65.

An example is described in relation to diabetes, wherein a treatment, involving mitochondrial DNA protection and other actions, collectively actions a) to j) can be designed by creating a compound, which is active at all of the sites a) to j).

An example is described on page 64 in relation to stimulation of insulin release in diabetes, wherein a treatment, involving Chromium and vanadium polyamine complexes is designed for that purpose.

The subsequent example describes a means of counteracting hyperinsulinism and obesity, wherein a treatment, involving a trivalent chromium polyamine complex is designed for that purpose.

The subsequent example describes a means of increasing insulin sensitivity in diabetes, wherein a treatment, involving collectively a) to l) can be designed by creating a vanadium polyamine complex, which is active at all of the sites a) to l).

The subsequent example describes a means of treating diabetic nephropathy, wherein a treatment, involving inhibition of protein kinase c is designed for that purpose.

The subsequent example describes a means of treating stroke wherein a treatment, involving mitochondrial DNA protection and other actions, collectively a) to l) can be designed by creating a compound, which is active at all of the sites a) to l).

The subsequent example describes a treatment for atherosclerosis, involving mitochondrial DNA protection and other actions, collectively a) to i) can be designed by creating a compound or vanadium metal complex or chromium metal complex which is active at all of the sites a) to i).

The subsequent example describes a treatment for glaucoma, involving mitochondrial DNA protection and other actions, collectively a) to j) can be designed by creating a compound which is active at all of the sites a) to j).

The subsequent example describes a treatment for presbycussis, involving mitochondrial DNA protection and other actions, collectively a) to 1) can be designed by creating a compound which is active at all of the sites a) to i).

The last example in that section describes a treatment for cancer in which a cobalt dihomocysteine polyamine complex which behaves like thioretinaco is utilized.

The biochemical criteria described on those pages represent the key biochemical defects and target control points for drug therapy of neurodegenerative and diabetic diseases. It has taken medical researchers enormous work in recent decades to discover their significance. The compositions and methods of design of multiple action compounds of this patent can be readily used to create optimal treatments. In the design of these compounds mitochondrial protection capability is always an a priori feature. Hence the physician is keen to obtain usable compounds which can regulate these mechanisms in selective groupings in order to more effectively treat his / her patients.

Manner of Deployment of the Instant Patent Application's Technology to Optimize Drugs and their Development

The medicinal chemist, pharmacologist, biochemist have an array of workhorse drug optimization techniques to utilize in optimizing these polyamine compounds to devise the most effective drug treatments.

It is very feasible using computational and bench assay techniques to identify such ideal drug discovery leads and further evaluate them in man pre and post market approval for particular disease indications.

In the past thirty years there has been a rapid and profound advancement in software programs from companies such as Accelrys, Tripos, Schroedinger and Open Eye, which have provided largely similar work horse techniques towards identifying efficacious and non toxic compounds for development as drugs for mammalian use. Further refinements by these companies and by academic practitioners in the field have provided approaches and solutions to

more advanced computational problems.

Each of the above companies provides a menu of programs which when operated on a parallel cluster of computers (a basic supercomputer) containing less than or more than one hundred nodes can design and analyze the performance of compounds in libraries, whose library size ranges from the tens of thousands to many millions of compounds. These analytic cycles of study each typically run over some hours to days.

The following is just one example choice of the use of a combination of some of these program components to identify efficacious polyamine compound towards the treatment of neurodegenerative and diabetic diseases, which could be devised and executed by ordinary computational medicinal chemists, and bench screening scientists and organic chemists.

The basic sequence in designing a mitochondrially protective drug and simultaneously designing the drug to include one or more additional useful actions towards treating a disease is to utilize data from an assay system such as that described in the parent U.S. Patent No. 5,906,996 to create a pharmacophore. A pharmacophore is a computational model of the structure of an active compound which defines the core chemical characteristics of size, shape and charge a molecule possesses in order to be an agonist or antagonist of a particular target site. Such a model may for example be computed following inputting assay data on four highly active compounds, four moderately active compounds, four lowly active compounds and four inactive compounds.

A library of compounds is built using the pharmacophore as the point of departure in designing the compounds. This library is screened for activity against the target(s). The universe of chemical space is further explored by creating sublibraries based upon compounds found to be

most active in earlier searches. Screening libraries may also be designed and subsequently analyzed without prior creation of a pharmacophore.

Where multiple activities are required in a single compound the library is screened against the multiple biochemical targets and in the manner of a Venn diagram the compounds showing highest activities for all the required targets are selected and utilized to create the next generations of sublibraries and ultimately to select the most efficacious compound.

Because of the diversity of structures which can be designed and the need for many activities in a single compound when treating these diseases, it is also possible to accelerate this design process by using rapid screening techniques such as the Topomer Program from Tripos Software, which by focusing on the active components of a compound and screening for interaction with the active site or the 3DPL program from LION Bioscience / Chem Navigator software which identifies active sites and screens compounds for activity against the surface of target macromolecules. Both of these techniques can screen a million compounds per day per computer node and they can serve as useful precursors, adjuncts or follow up to conventional pharmacophore and structure activity based library design and screening, particularly where optimizing compounds to include and or exclude multiple activities is intended.

Computational screening can also include assessment of compounds for their likely absorption, tissue distribution, metabolism and excretion (ADME) and safety characteristics.

In vitro screening assays can frequently be purchased from commercial vendors and are utilized in the computational design, chemical synthesis and screening program to yield the most suitable drug candidates to treat particular diseases and complications thereof. The results of in vitro assays may be used to facilitate initial design of pharmacophore, to verify the results of

computational screening.

Following is a more detailed description of three example tasks and subtasks such a drug optimization process can include which applies to polyamines of the various families the Applicant and his associate synthesized as disclosed in the instant patent application and its associated continuations Composition, Synthesis and Therapeutic Application of Polyamines December 18, 2002 filed as a P.C.T. application PCT/US02/40732 with domestic re entry in the U.S. Patent Application No. 10/499,931. This description is merely by way of example and is not meant to be limiting, since each ordinary practitioner may choose whichever programs components from the major computational software vendors and public domain sources they wish or as are available to them. The three tasks are:

a) molecular design

b) synthesis of molecules

c) evaluation of the efficacy of the molecules by biochemical methods and animal models

Introduction

Applicant and his staff extended their study on the targets using structure based drug design by amplification of the existing polyamine compounds which were most active i.e. activity at micromolar and lower dose range in mitochondrial protection. The protocol includes design of pharmacophores by utilizing commercial software. These steps are followed by building the analogs in silico, docking them at the active sites and deriving the optimal structures in terms of energy of association scores. These libraries of compounds are further verified by

entropy and toxicity calculations. This approach reduces the number of molecules, which need be synthesized. The molecules are synthesized by manual (conventional chemistry) and parallel synthesis. These compounds are tested in vitro by high throughput screening and in vivo by animal modeling. This methodology explores the quantitative structure activity relationship as observed from the physical, chemical and biological properties of the compounds and their agonistic / antagonistic actions. This procedure leads to development of a better pharmacophore for an iterative approach, resulting in compounds efficacious at pico or nano or micro molar doses.

Tasks

Task 1.0 Molecular Design. Extensive literature search, both using online and offline methods to derive in depth knowledge of the target molecules and their physical /chemical characterization and the arrangement of active site in the target macromolecules. The efficacy of small molecules is recorded. Successful completion of the search is our milestone and the metric for this task is the identification of the small molecule agonists or antagonists.

Task 1.02 The three dimensional structures of the compounds are derived using standard energy minimization protocols. They are pooled with our existing families of polyamine compounds. Then using comparative study procedures like GASP are used to arrive at pharmacophores. The milestone of this task is to derive at least a few distinct pharmacophores and the metric is the number of useful pharmacophores.

Task 1.03 The designed pharmacophores represent our initial seven polyamine families combined with the information obtained from the literature search (Task 1.01). These

parmacophores are expanded by creating polyamines with behave similarly to the polyamine vanadium and polyamine chromium complexes. The programs such as Select and CombiLib are used to build these analogs. The metric is the creation of a library of 50,000 compounds from this task.

Task 1.04 These analogs are filtered by initial screening methods, including the measurement of C log P to provide information on solubility and membrane permeability. Molecules not passing these filters are removed from the library. The program SBF (Structure Based Focusing) developed by Accelrys is used to eliminate the compounds, which are not viable, as drug compounds in terms of surface features. It is expected that ten percent will be eliminated by this procedure. The milestone is to truncate the library and the metric is ten percent reduction in library size.

Task 1.05 This selected library of compounds are docked at the binding site of the target molecules to derive the energetics of binding. This procedure is highly critical though it requires much computational time the Applicant adopted three different docking procedures to derive the optimum binding of these molecules in terms of charge, size, shape and hydrophobicity. These docking procedures are AutoDoc (developed at The Scripps Research Institute), Ligand Fit (Accelrys) and Score (Sybil). The Applicant also devised a special procedure to order the compounds in terms of their affinity to the binding site from the docking procedure of these three programs. The computationally intensive procedure ranks the compounds by affinity. The Applicant select the five hundred most promising compounds from these screens. Milestone is the successful completion of docking and the metric is the ranking.

Task 1.06 These compounds are further tested in terms of their entropic contribution.

The surface accessible area of the free molecule, active site and complex of the active site with the free molecule is calculated using the program MSMS (developed at The Scripps Research Institute). The loss of both polar and non-polar areas is calculated and the entropy of the interaction is derived using standard formulas. These results reassign the ranking index from the previous task. The completion is a milestone and the reassignment is a metric.

Task 1.07 These compounds are further filtered for toxicity. The chemical structures of these compounds are converted to SMILES format and tested for toxicity by using the program developed by E.P.A. The successful completion of this task including the format conversion is a milestone. The rate of elimination from the library is typically approximately ten to fifteen percent. However the Applicant still wanted to rank high affinity compounds which are expected to be potent agonists or antagonists and whose toxicity may be diminished by substitution or elimination of specific elements.

The final goal of Tasks 1.0-1.07 is to provide a list of compounds for synthesis and in vitro evaluation.

Task 2.0 These families of compounds are synthesized by manual and parallel procedures. The synthetic routes have been developed by us previously. The duplication of the previous efforts is avoided. Preference is given to the compounds selected during task one. However adequate care must be taken to minimize the number of synthetic steps to achieve higher yields. The milestone of this task is to synthesize seventy percent of the compounds selected during task one and the metric is three hundred and fifty compounds.

Task 2.02 These compounds are purified by high performance liquid chromatography and fast performance liquid chromatography. The compounds are tested for their chemical

nature and intactness by mass spectroscopy and one dimensional NMR. The milestone of this task is to obtain pure compounds and the metric is three hundred and fifty compounds. These compounds are further characterized by Raman and Infra Red measurements.

The milestone of Tasks 2.0-2.02 is to synthesize a compound library of three hundred and fifty, which are well characterized and well tested for homogeneity.

Task 3.0 Biochemical Evaluation

Task 3.1 Biochemical Method

Most of the receptors are available commercially. The association kinetics are studied by standard biochemical methods to derive IC 50 values. The milestone is the completion of assays and the metric is to record IC 50 values.

Task 3.2 Animal Models

Efficacy studies in prevention and or recovery experiments are done in small animal models and pharmacokinetics are also studied. Statistically significant results are sought in these tried and tested models.

Task 4.0 QSAR (Quantitative Structure Activity Relationship)

The results from the experimental studies are correlated with the molecular structure of the compounds, and the contribution of the individual constituents is derived from this investigation. Both linear and non-linear correlation between the experimental results and constituents of the structure and their physical and chemical properties is derived by a multi correlation analysis. These results are used to derive a new pharmacophore as well as to improve the existing compounds. The successful completion of QSAR calculation is the milestone and the classification of the contribution of each constituent.

Task 4.1 Dàta for Second Iteration

The entire results are analyzed and decisions will be reached regarding the next iteration or recommendation for preparation of IND.

Interrelationship of Tasks

These tasks are closely interrelated. The first task is to design a library of compounds which itself is based upon our earlier library of synthesized compounds. Our rigorous computational protocol would recommend the compounds for synthesis. These design efforts result from free and frank discussion between synthetic chemists and computational chemists. During the synthesis computational chemists are consulted with on the design of synthetic routes. After the synthesis, these compounds are evaluated for their potency by biochemical methods. The assay results are interpreted by the computational chemists, chemists and biologists to generate further refinements of the compounds and thus more syntheses. The entire iterative procedure is interrelated.

Milestones and Metrics

task	Milestone	Metric
1.0	Successful completion of the literature search	Identification of the small molecule agonists or antagonists.
1.02	Derive at least a few distinct pharmacophores	Number of useful pharmacophores
1.03	Analog building completion	Creation of a library of 50,000 compounds
1.04	Truncate the library of compounds	Ten percent reduction in library size.
1.05	Successful completion of docking	Affinity ranking of compounds
1.06	Completion of entropic calculation	Reassignment of Affinity ranking
1.07	List of compounds for synthesis	High affinity compounds
2.0	Synthesize seventy percent of the compounds	Synthesize three hundred fifty compounds
2.02	Obtain pure compounds by purification methods	Three hundred and fifty compounds
3.1	Completion of Assay with receptors	IC 50 values
3.2	Completion of Animal Models	Statistically significant efficacy and bioavailability
4.0	QSAR (Quantitative Structure Activity Relationship)	Classification of the contribution of each constituent

These tasks are repeated during each iteration, and multiple targets are done concurrently. In some instances the Topomer and 3DPL programs are utilized to define the best chemical space for library building. These programs not only confer high speed in screening compounds, they also provide different level of view, wherein the topomer technique looks at a portion of an active site, conventional docking looks at the whole active site and 3DPL looks at the active site in context of its position in the macromolecule. Under optimal operating circumstances the Applicant identified individual Parkinson, Alzheimer, Lou Gehrig's, stroke, cardiomyopathy,

atherosclerosis, type I diabetes, type II diabetes, maturity onset diabetes of the young, glaucoma, presbycussis and nephropathy drug candidates in months.

Upon identification of these lead compounds animal safety is evaluated prior to commencing clinical trials in man.

Toxicology, Safety Trials and Efficacy Clinical Trials in Man

In summary a range of drugs with effective combined therapeutic profiles are designed to treat a range of mitochondrial diseases and developed based upon structure based drug design. Their subsequent evaluation includes:

In vivo animal studies in a few species for activity in vivo;

Process chemistry to facilitate scale up synthesis alongside appropriate formulation; Toxicologic and safety studies in vitro and in vivo including studies of absorption, distribution, metabolism and excretion (ADME).

The primary screening is based upon designing compounds which were effective in protecting against mitochondrial DNA damage, followed by therapeutic predictors such as the nineteen therapeutic actions in Parkinson's disease and Alzheimer's disease or a subgroup of therapeutic actions to treat Type I diabetes, Maturity Onset Diabetes of the Young (MODY), Type II diabetes, diabetic nephropathy, diabetic cardiomyopathy, diabetic peripheral neuropathy and other diabetic variants and complications. Such therapeutic mechanisms in treating a diabetic disease or neurodegenerative disease could include or exclude any of forty nine activities previously recognized as being displayed by polyamines. As with mitochondrial neurodegenerative diseases a great many of these polyamine activities are linked to common

biochemical defects occurring during the progression of diabetic diseases in the biomedical literature. These include for example tyrosine phosphatase, protein kinase C, PPARα and PPARγ.

Secondary screening includes study of whole cell models such as insulin release, ion flux and electrophysiology. Also undesirable effects such as inhibition of ornithine decarboxylase (natural polyamine synthetic enzyme) or inhibition of the polyamine transporter need to be excluded in any drug treating neurodegenerative or diabetic diseases.

Process Chemistry includes scale up development for synthesis of Kilogram and greater quantities of drug under GLP and GMP conditions. This includes identification of metabolic impurities. Further formulations for oral and other routes of delivery and delivery in relation to consumption of other drugs, food, alcohol will be studied throughout the preclinical and clinical trial stages of drug development. Drug products may include conventional and slow release formulations.

A pharmaceutical profile is built by studies of mutagenesis using the Ames Test, absorption and passive diffusion using CACO-2 Cells, metabolic stability using rat hepatosomes, lipophilicity LogD/LogP, drug interactions using CYP isoforms and plasma protein binding. In vitro metabolism is studied using muscle, liver microsomes and whole blood are performed. These in vitro screens already exist.

The toxicity and therapeutic efficacy of the compounds is also determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the L.D. 50 (the dose lethal to 50% of the population) and the E.D. 50 (the dose therapeutically effective

in 50% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index and can be expressed as the ratio between L.D. 50 and E.D. 50. Compounds, which exhibit high therapeutic indices, are preferred. The data obtained from these cell culture assays and animal studies is used in formulating a dosage range that is not toxic for use in man. The dosage of such compounds lies preferably within a range of circulating concentrations that include the E.D. 50 with little or no toxicity.

In vivo screens study dosing regimes and efficacy in small animal models, whole animal pKa in rats and radiolabelling studies of drug distribution. These studies of Absorption, Distribution, Metabolism and Excretion (ADME) in rat and dog facilitate developing a dosing schedule for use in human clinical trials. Plasma half life needs to be appropriate for continuous therapeutic effect.

Previous oral, systemic, inhalational and dermatological exposure of animals and man to polyamines has demonstrated overall good safety characteristics. Acute toxicity such as maximum tolerated dose is studied in rats and formal GLP toxicology including week, two week and four exposure in rat and dog is done prior to filing an investigational new drug application (IND).

Thus a product profile, which in many instances will be single daily oral drug therapy to treat a variety of disease will be created by using screens and processes which already exist, resulting in low toxicity drugs with moderate or long half lives.

Clinical trials are effective in phases or expanded trials and a product label designed for subsequent new drug approval submission to FDA and other regulatory agencies.

Clinical trials vary considerably in numbers of patients and duration of trials to demonstrate efficacy of the compounds for respective diseases in man. Conventionally they extend through three phases. Phase I trials establish safety on single and multiple doses in man. Phase II demonstrate efficacy against disease and Phase III trials establish efficacy at different dosage and timing schedules in different populations and in relation to use of other drugs, alcohol and dietary variations. Phase I/IIa trials are sometimes performed in an affected disease group rather then conducting separate Phase I trials in a disease free population. Single trial drug development is also possible by ballooning the patients from earlier phases into the later stages. Most trials are conducted as double blind placebo controlled trials in which investigators and patients are not informed of whether the individual patients are receiving active drug or placebo until a period of time in which to evaluate clinical effect has passed. Some trials are internally controlled by placing patients on drug for a period and on placebo for another period, which sequence of periods is randomly assorted and blinded. Bayesian statistical analysis can also be used to reach earlier statistical decision points in trials and it is particularly useful in situations where the treatment group is characterized by all of its members having a particular genetic or biochemical defect, in which situations all of the affected persons should respond to a treatment rather than merely a subgroup.

As with computational and in vitro screening the methods of clinical trials, the methodologies are laborious, tried and tested and within the range of ordinary scientific and clinical practitioners to reach conclusions of successful clinical efficacy without being reinvented. For example in Parkinson's disease the performance of patients on the United Parkinson's Disease Rating Scale (UPDRS) compares the efficacy of a proposed treatment versus

none or currently in use symptomatic treatment(s) such as levodopa. In diabetes control of glucose tolerance alongside diminishing the occurrence of cardiac and neurologic complications is evaluated in comparison with the efficacy of an existing gold standard drug such as Metformin. Biochemical measurements can be voluminous in the assessment of drugs to treat diabetes and its complications and may include new genetic and biochemical assays focused on some of the more specific biochemical opportunities I have identified here.

The prescribing of these drugs will be determined by the uses approved by the regulatory agencies. In general it is anticipated that treatment will be used long term and that the oral route of delivery will be used predominantly.

Formulation - Dosage, Chemical Forms, Routes of Delivery and Pharmaceutical Compositions

Dosage

The amount of active compound that can be combined with a carrier material to produce a single dosage form will vary depending upon the disease treated, the mammalian species, the potency of the active component and the particular mode of administration. The level of dosage to treat a disease is determined by the concentration of drug required to cause efficacy at the receptor at which the drug has least efficacy amongst the group of target receptors being treated by the drug.

However, as a general guide, suitable unit doses for the compounds of the present invention can, for example, preferably contain between 1 mg to about 3000 mg of the active

compound. A preferred unit dose is between 1 mg to about 500 mg. A more preferred unit dose is between 1 to about 90 mg. Such unit doses can be administered more than once a day, but preferably 1 or 2 times per day, so that the total daily dosage for a 66 kg adult is in the range of 0. 01 to about 45 mg per kg weight of subject per administration. A preferred dosage is 0.01 to about 7.5 mg per kg weight of subject per administration, and such therapy can extend for weeks, months or years. For topical administration, dosages in the range of about 0.05-20% concentration of the compound, preferably 1-5%, are suggested.

The dosage of each agent will vary depending upon the severity of the disease, the frequency of administration, the particular agent utilized, and other factors routinely considered by an attending medical practitioner.

It is contemplated that, as part of their patient evaluations, the attending physicians know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physicians also know when to adjust treatment to higher levels, in circumstances where the clinical response is inadequate, while precluding toxicity. The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated, the patient's individual physiology, biochemisty and the route of administration. The severity of the condition, may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and dose frequency will also vary according to the age, body weight, sex and response of the individual patient.

Chemical forms

Certain of the compounds of the present invention can exist in unsolvated forms as well as solvated forms, including, but not limited to, hydrated forms. In general, the solvated forms, including hydrated forms, are equivalent to unsolvated forms. Certain of the compounds of the present invention possess one or more chiral centers and each center may exist in different configurations.

As generally understood by those skilled in the art, an optically pure compound having one chiral center (i.e., one asymmetric carbon atom) is one that consists essentially of one of the two possible enantiomers (i.e., is enantiomerically pure), and an optically pure compound having more than one chiral center is one that is both diastereomerically pure and enantiomerically pure. Preferably, the compounds of the present invention are used in a form that is at least 90% optically pure, that is, a form that contains at least 90% of a single isomer (80% enantiomeric excess ("e.e.") or diastereomeric excess ("d.e.")), more preferably at least 95% (90% e.e. or d.e.), even more preferably at least 97.5% (95% e.e. or d.e.), and most preferably at least 99% (98% e.e. or d.e.).

Where stereospecific synthesis techniques are employed or optically active compounds are employed as starting materials in the preparation of the compounds, individual isomers may be prepared directly. However, if a mixture of isomers is prepared, the individual isomers may be obtained by conventional resolution techniques, or the mixture may be used as is, with resolution. Furthermore, some of the compounds can exist in the form of tautomeric isomers.

Routes of Delivery and Pharmaceutical Compositions

The compounds that are used in the methods of the present invention can be incorporated into a variety of formulations for therapeutic administration. More particularly, the can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and can be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, pills, powders, granules, dragees, gels, slurries, ointments, solutions, suppositories, injections, inhalants and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intranasal, intracranial, intraaural, intraocular or intratracheal administration. Moreover, the compound can be administered in a local rather than systemic manner, in a depot or sustained release formulation. In addition, the compounds can be administered in a liposome.

Pharmaceutically acceptable acid addition salts of the compounds of the invention which contain basic groups are formed where appropriate with strong or moderately strong, non-toxic, organic or inorganic acids in the presence of the basic amine by known methods. Examples of the acid addition salts are maleate, fumarate, lactate, oxalate, methanesulfonate, ethanesulfonate, benzenesulfonate, tartrate, citrate, hydrochloride, hydrobromide, sulfate, phosphate and nitrate salts.

Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline, water, dextrose, glycerol and the like. Similarly, the carrier or diluent

may include any prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft gelatin capsule, liquid containing capsule, sterile injectable liquid (e.g., a solution), such as an ampoule, or an aqueous or nonaqueous liquid suspension. A summary of such pharmaceutical compositions may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton PA. (Gennaro 18th ed. 1990).

Dosage forms of the compositions of the present invention can also be formulated as enteric coated delayed release oral dosage forms, i.e., as an oral dosage form of a pharmaceutical composition as described herein which utilizes an enteric coating to effect release in the lower gastrointestinal tract. The enteric coated dosage form may be a compressed or molded or extruded tablet / mold (coated or uncoated) containing granules, pellets, beads or particles of the active ingredient and / or other composition components, which are themselves coated or uncoated. The enteric coated oral dosage form may also be a capsule (coated or uncoated) containing pellets, beads or granules of the solid carrier or the composition, which are themselves coated or uncoated.

The term "delayed release" as used herein refers to the delivery so that the release can be accomplished at some generally predictable location in the lower intestinal tract more distal to that which would have been accomplished if there had been no delayed release alterations. The preferred method for delay of release is coating. Any coatings should be applied to a sufficient thickness such that the entire coating does not dissolve in the gastrointestinal fluids at pH below about 5, but does dissolve at pH about 5 and above. It is expected that any anionic polymer

exhibiting a pH-dependent solubility profile can be used as an enteric coating in the practice of the present invention to achieve delivery to the lower gastrointestinal tract.

Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are known. Sustained-release capsules may, depending on their chemical nature, release the compounds for weeks to months.

Also prodrugs and active metabolites of such compounds, and pharmaceutically acceptable salts of such metabolites may be used. A "pharmaceutically acceptable prodrug" is a compound that may be converted under physiological conditions or by solvolysis to the specified compound or to a pharmaceutically acceptable salt of such compound. A "pharmaceutically active metabolite" is a pharmacologically active product produced through metabolism in the body of a specified compound or salt thereof.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are known examples of delivery vehicles or carriers for hydrophobic drugs.

Conclusion

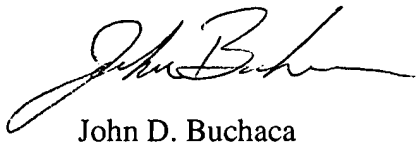
This invention demonstrates compositions and utility of polyamines in treating acquired mitochondrial DNA damage diseases, therapeutic avenues for a range of disease subsets and complications by designing drugs with specifically selected multiple sites of action, opportunities for medicinal chemists following conventional computational screening, synthetic and assay techniques to identify the most active drug compounds and for clinicians to establish their

efficacy in clinical trials. It is evident that the novel opportunities for more specific and comprehensive treatments are extensive across the two groups of disease, neurodegenerative and diabetic, which comprise acquired mitochondrial diseases. The capacity of polyamines in preventing mitochondrial damage and treating mitochondrial diseases was not obvious to a person of "ordinary skill" in the field prior to the discovery presented in parent U.S. Patent No. 5,906,996 and the instant patent application.

The tasks required to develop these products and the selection of the activity characteristics and actions to exclude are within the scope of practice of ordinary scientific and medical practitioners in the field. Without the demonstration of the efficacy of polyamines in preventing mitochondrial damage in this application and its parent, scientific and medical practitioners would be unaware of these opportunities for more effective treatments of neurodegenerative and diabetic diseases using polyamines. Frequently a drug developer will have many choices of compound families, which exert a similar biological action, from which to choose a future drug. However the compound family he / she wishes to utilize will be that which is non toxic or related to an initial step in the disease process, as distinct to a randomly effective compound. It is readily apparent how vast the impact on quality and duration of life recognition of this choice of mitochondrially protective compounds in developing drugs for the neurodegenerative and diabetic diseases can be and that this opportunity coupled with the opportunity of acting at multiple target sites is not apparent in any other class of molecule.

In view of the above, allowance of the pending claims is earnestly solicited.

Respectfully submitted,



John D. Buchaca
Registration No. 37,289

1545 Hotel Circle South, Suite 150
San Diego, California 92108-3426
Telephone: (619) 294-2922
Fax No. (619) 294-8674

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